■ РЕФЕРАТИ СТАТЕЙ, ОПУБЛІКОВАНИХ В «CYTOLOGY AND GENETICS», № 4, 2020 р.

NOVEL MUTATION C.7348C>T IN NF1 GENE IDENTIFIED BY WHOLE-EXOME SEQUENCING IN PATIENT WITH OVERLAPPING CLINICAL SYMPTOMS OF NEUROFIBROMATOSIS TYPE 1 AND BANNAYAN-RILEY-RUVALCABA SYNDROME

E.S. RAHMANI [1], H. AZARPARA [2], M.F. ABAZARI [3], M.R. MOHAJERI [1], M. NASIMI [4], R. GHORBANI [5], A. AZIZPOUR *[4], H. RAHIMI *[5]

[1] Department of Medical Biotechnology, School of Allied Medicine, Iran University of Medical Science, Tehran, Iran
[2] Department of Medicine, Iran University of Medical Science, Tehran, Iran
[3] Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
[4] Department of Dermatology Razi Hospital, Tehran University of Medical Science, Tehran, Iran
[5] Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
E-mail: rahimi.h1981@gmail.com

Neurofibromatosis type 1 (NF-1) is an autosomal dominant disorder provoking benign cutaneous and nerve sheath tumors. The cutaneous tumors termed as plexiform neurofibromas, which some of them are extremely visible, and can influence the quality of life. They can also develop into invasive forms of carcinomas and infiltrate into multiple tissues, thus endangering the patient's life. The loss-of-function mutations in NF1 gene are responsible for NF-1 type. Due to the large size of NF1 gene (~ 350 kb and 60 exons), exist some pseudogenes on another locus, and lack mutation hotspot the molecular characterizing of patients is complex. In this study, we reported a patient showed symptoms of both NF-1 and Bannayan-Riley-Ruvalcaba syndrome (BRRS), then performed a whole-exome sequencing (WES) and a data analysis for molecular characterization. These results showed a single heterozygous nucleotide variant (c.7348C>T) in NF1 gene, which results in a premature stop codon (p.Arg2450Ter) and a truncated protein, causing clinical symptoms of the patient. According to the results, WES is a quick and cost-effective approach for molecular diagnosis of the mixed phenotype of NF-1.

Key words: *Neurofibromatosis type 1(NF-1); Bannayan-Riley-Ruvalcaba syndrome (BRRS); Whole-exome sequencing (WES); Neurofibromin; Bioinformatics; Molecular diagnosis*

ІДЕНТИФІКАЦІЯ НОВОЇ МУТАЦІЇ C.7348C>T ГЕНУ NF1 ЗА ДОПОМОГОЮ ПОВНОЕКЗОМНОГО СЕКВЕНУВАННЯ У ПАЦІЄНТА З СУПУТНІМИ КЛІНІЧНИМИ ОЗНАКАМИ НЕЙРОФІБРОМАТОЗУ 1 ТИПУ І СИНДРОМУ БАННАЯН-РАЙЛІ-РУВАЛЬКАБА

Нейрофіброматоз 1 типу (NF-1) – це аутосомне домінантне захворювання, яке призводить до виникнення доброякісних пухлин на шкірі та в оболонках нервів. Шкірні пухлини називають плексиформними нейрофібромами. Деякі з них добре видно неозброєним оком і вони можуть впливати на якість життя. Також вони можуть розвиватися в інвазивні форми карцином та інфільтрувати у багато тканин, чим ставлять життя пацієнта під загрозу. Мутації гену NF1 з втратою функції відповідають NF-1 типу. Через великий розмір гену NF1 (~ 350 кб і 60 екзонів) на іншому локусі існують деякі псевдогени; молекулярна характеристика пацієнтів ускладнена відсутністю «гарячої точки» мутації. У цьому дослідженні ми повідомляємо про пацієнта, який мав симптоми і NF-1, і синдрому Баннаяна-Райлі-Рувалькаба (BRRS), а також про наше проведення повноекзомного секвенування (WES) і аналізу даних для молекулярної характеристики. Ці результати показали одиничний гетерозиготний нуклеотидний варіант (c.7348C>T) гену NF1, який викликав передчасний стоп-кодон (p.Arg2450Ter) та процесований білок, чим спричинив клінічні ознаки пацієнта. Згідно з результатами, WES – це швидкий та малозатратний підхід до молекулярної діагностики змішаного фенотипу NF-1.

Ключові слова: нейрофіброматоз типу 1(NF-1), синдром Баннаян-Райлі-Рувалькаба (BRRS), повноекзомне секвенування (WES), нейрофібромін, біоінформатика, молекулярний діагноз.

REFERENCES

1. Friedman, J., Review article: Neurofibromatosis 1: Clinical manifestations and diagnostic criteria, *J. Child. Neurol.*, 2002, vol. 17, no. 8, pp. 548–54.
2. Wiest, V., Eisenbarth, I., C. Schmegner, Krone, W., and Assum, G., Somatic NF1 mutation spectra in a family with neurofibromatosis type 1: toward a theory of genetic modifiers, *Hum. Mutat.*, 2003, vol. 22, no. 6, pp. 423–7.
3. Gutmann, D.H., Ferner, R.E., Listernick, R.H., Korf, B.R., Wolters, P.L., and Johnson, K.J., Neurofibromatosis type 1, *Nat. Rev. Dis. Primers*, 2017, vol. 3, pp. 17004. doi: 10.1038/nrdp.2017.4.
4. McClatchey, A.I., Neurofibromatosis, *Annu. Rev. Pathol. Mech. Dis.*, 2007, vol. 2, pp. 191–216.
5. Monroe, C.L., Dahiya, S., and Gutmann, D.H., Dissecting Clinical Heterogeneity in Neurofibromatosis Type 1, *Annu. Rev. Pathol. Mech. Dis.*, 2017, vol. 12, no. 1, pp. 53–74. doi: 10.1146/annurev-pathol-052016-100228.
6. Williams, V.C., Lucas, J., Babcock, M.A., Gutmann, D.H., Korf, B., and Maria, B.L., Neurofibromatosis type 1 revisited, *Pediatrics*, 2009, vol. 123, no. 1, pp. 124–33.
7. Chen, H., *Bannayan-Riley-Ruvalcaba Syndrome*, New York, NY: Springer New York, 2016.
8. McKeever, K., Shepherd, C.W., Crawford, H., and Morrison, P.J., An epidemiological, clinical and genetic survey of neurofibromatosis type 1 in children under sixteen years of age, *Ulster Med. J.*, 2008, vol. 77, no. 3, pp. 160–3.
9. Rojnueangnit, K., Xie, J., Gomes, A., Sharp, A., Callens, T., Chen, Y., Liu, Y., Cochran, M., Abbott, M.A., and Atkin, J., High incidence of Noonan syndrome features including short stature and pulmonic stenosis in patients carrying NF1 missense mutations affecting p. Arg1809: genotype–phenotype correlation, *Hum. Mutat.*, 2015, vol. 36, no. 11, pp. 1052–63.
10. Upadhyaya, M., Huson, S.M., Davies, M., Thomas, N., Chuzhanova, N., Giovannini, S., Evans, D.G., Howard, E., Kerr, B., and Griffiths, S., An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c. 2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation, *Am. J. Hum. Genet.*, 2007, vol. 80, no. 1, pp. 140–51.
11. Cawthon, R.M., Weiss, R., Xu, G., Viskochil, D., Culver, M., Stevens, J., Robertson, M., Dunn, D., Gesteland, R., and O'Connell, P., A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations, *Cell*, 1990, vol. 62, no. 1, pp. 193–201.
12. Masocco, M., Kodra, Y., Vichi, M., Conti, S., Kanieff, M., Pace, M., Frova, L., and Taruscio, D., Mortality associated with neurofibromatosis type 1: a study based on Italian death certificates (1995–2006), *Orphanet J. Rare Dis.*, 2011, vol. 6, no. 1, pp. 11.
13. Schreibman, I.R., Baker, M., Amos, C., and McGarrity, T.J., The hamartomatous polyposis syndromes: a clinical and molecular review, *Am. J. Gastroenterol.*, 2005, vol. 100, no. 2, pp. 476–90.
14. Jett, K., Friedman, J.M., Clinical and genetic aspects of neurofibromatosis 1, *Genet. Med.*, 2010, vol. 12, no. 1, pp. 1–11.
15. Xuan, J., Yu, Y., Qing, T., Guo, L., and Shi, L., Next-generation sequencing in the clinic: promises and challenges, *Cancer Lett.*, 2013, vol. 340, no. 2, pp. 284–95.
16. Faden, D.L., Asthana, S., Tihan, T., DeRisi, J., and Kliot, M., Whole exome sequencing of growing and non-growing cutaneous neurofibromas from a single patient with neurofibromatosis type 1, *PLoS ONE*, 2017, vol. 12, no. 1, pp. e0170348.
17. McPherson, J.R., Ong, C.K., Ng, C.C.Y., Rajasegaran, V., Heng, H.L., Yu, W.S.S., Tan, B.K.T., Madhukumar, P., Teo, M.C.C., and Ngeow, J., Whole-exome sequencing of breast cancer, malignant peripheral nerve sheath tumor and neurofibroma from a patient with neurofibromatosis type 1, *Cancer Med.*, 2015, vol. 4, no. 12, pp. 1871–8.
18. Li, H., Durbin, R., Fast and accurate short read alignment with Burrows–Wheeler transform, *Bioinformatics*, 2009, vol. 25, no. 14, pp. 1754–60.
19. Zhao, X., Wang, A., Walter, V., Patel, N.M., Eberhard, D.A., Hayward, M.C., Salazar, A.H., Jo, H., Soloway, M.G., and Wilkerson, M.D., Combined targeted DNA sequencing in non-small cell lung cancer (NSCLC) using UNCseq and NGScopy, and RNA sequencing using UNCqeR for the detection of genetic aberrations in NSCLC, *PLoS ONE*, 2015, vol. 10, no. 6, pp. e0129280.
20. Robinson, J.T., Thorvaldsdyttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and Mesirov, J.P., Integrative genomics viewer, *Nat. Biotechnol.*, 2011, vol. 29, no. 1, pp. 24–6.
21. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., and Daly, M., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data, *Genome Res.*, 2010, vol. 20, no. 9, pp. 1297–303.
22. 22. Eppig, J.T., Blake, J.A., Bult, C.J., Kadin, J.A., Richardson, J.E., and Group, M.G.D., The Mouse Genome Database (MGD): facilitating mouse as a model for human biology and disease, *Nucl. Acids Res.*, 2015, vol. 43, no. D1, pp. D726–D736.
23. Firth, H.V., Richards, S.M., Bevan, A.P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R.M., and Carter, N.P., DECIPHER: database of chromosomal imbalance and phenotype in humans using ensembl resources, *Am. J. Hum. Genet.*, 2009, vol. 84, no. 4, pp. 524–33.

24. Huang, D.W., Sherman, B.T., and Lempicki, R.A., Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists, *Nucl. Acids Res.*, 2008, vol. 37, no. 1, pp. 1–13.
25. Ng, P.C., Henikoff, S., SIFT: Predicting amino acid changes that affect protein function, *Nucl. Acids Res.*, 2003, vol. 31, no. 13, pp. 3812–4.
26. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R., A method and server for predicting damaging missense mutations, *Nat. Meth.*, 2010, vol. 7, no. 4, pp. 248–9.
27. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J., A general framework for estimating the relative pathogenicity of human genetic variants, *Nat. Genet.*, 2014, vol. 46, no. 3, pp. 310.
28. Kanehisa, M., Goto, S., KEGG: kyoto encyclopedia of genes and genomes, *Nucl. Acids Res.*, 2000, vol. 28, no. 1, pp. 27–30.
29. Finn, R.D., Attwood, T.K., Babbitt, P.C., Bateman, A., Bork, P., Bridge, A.J., Chang, H.-Y., Dosztбnyi, Z., El-Gebali, S., and Fraser, M., InterPro in 2017–beyond protein family and domain annotations, *Nucl. Acids Res.*, 2016, vol. 45, no. D1, pp. D190-D199.
30. Beck, T.F., Mullikin, J.C., Biesecker, L.G., and Program, N.C.S., Systematic evaluation of Sanger validation of next-generation sequencing variants, *Clin. Chem.*, 2016, vol. 62, no. 4, pp. 647–54.
31. Wallace, M.R., Marchuk, D.A., Andersen, L.B., Letcher, R., Odeh, H.M., Saulino, A.M., Fountain, J.W., Brereton, A., Nicholson, J., and Mitchell, A.L., Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients, *Sci.*, 1990, vol. 249, no. 4965, pp. 181–7.
32. Clementi, M., Barbujani, G., Turolla, L., and Tenconi, R., Neurofibromatosis-1: a maximum likelihood estimation of mutation rate, *Hum. Genet.*, 1990, vol. 84, no. 2, pp. 116–8.
33. Pasmant, E., Parfait, B., Luscan, A., Goussard, P., Briand-Suleau, A., Laurendeau, I., Fouveaut, C., Leroy, C., Montadert, A., and Wolkenstein, P., Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations?, *Eur. J. Hum. Genet.*, 2015, vol. 23, no. 5, pp. 596–601.
34. Ingram, D.A., Yang, F.-C., Travers, J.B., Wenning, M.J., Hiatt, K., New, S., Hood, A., Shannon, K., Williams, D.A., and Clapp, D.W., Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumor suppressor gene modulates melanocyte and mast cell fates in vivo, *J. Exp. Med.*, 2000, vol. 191, no. 1, pp. 181–8.
35. Zhu, Y., Ghosh, P., Charnay, P., Burns, D.K., and Parada, L.F., Neurofibromas in NF1: Schwann cell origin and role of tumor environment, *Sci.*, 2002, vol. 296, no. 5569, pp. 920–2.
36. Thomas, L., Kluwe, L., Chuzhanova, N., Mautner, V., and Upadhyaya, M., Analysis of NF1 somatic mutations in cutaneous neurofibromas from patients with high tumor burden, *Neurogenet.*, 2010, vol. 11, no. 4, pp. 391–400.
37. Monroe, C.L., Dahiya, S., and Gutmann, D.H., Dissecting Clinical Heterogeneity in Neurofibromatosis Type 1, *Annu. Rev. Pathol. Mech. Dis.*, 2017, vol. 12, no., pp. 53–74.
38. Kluwe, L., Friedrich, R.E., Korf, B., Fahsold, R., and Mautner, V.F., NF1 mutations in neurofibromatosis 1 patients with plexiform neurofibromas, *Hum. Mutat.*, 2002, vol. 19, no. 3, pp. 309. doi: 10.1002/humu.9018.
39. De Schepper, S., Maertens, O., Callens, T., Naeyaert, J.-M., Lambert, J., and Messiaen, L., Somatic mutation analysis in NF1 cafй au lait spots reveals two NF1 hits in the melanocytes, *J. Invest. Dermatol.*, 2008, vol. 128, no. 4, pp. 1050–3.
40. Boyd, K.P., Gao, L., Feng, R., Beasley, M., Messiaen, L., Korf, B.R., and Theos, A., Phenotypic variability among cafй-au-lait macules in neurofibromatosis type 1, *J. Am. Acad. Dermatol.*, 2010, vol. 63, no. 3, pp. 440–7.
41. Vandenbroucke, I., Van Oostveldt, P., Coene, E., De Paepe, A., and Messiaen, L., Neurofibromin is actively transported to the nucleus, *FEBS Lett.*, 2004, vol. 560, no. 1–3, pp. 98–102.
42. Li, C., Cheng, Y., Gutmann, D.A., and Mangoura, D., Differential localization of the neurofibromatosis 1 (NF1) gene product, neurofibromin, with the F-actin or microtubule cytoskeleton during differentiation of telencephalic neurons, *Dev. Brain. Res.*, 2001, vol. 130, no. 2, pp. 231–48.
43. Lange, A., Mills, R.E., Lange, C.J., Stewart, M., Devine, S.E., and Corbett, A.H., Classical nuclear localization signals: definition, function, and interaction with importin α, *J. Biol. Chem.*, 2007, vol. 282, no. 8, pp. 5101–5.
44. Koliou, X., Fedonidis, C., Kalpachidou, T., and Mangoura, D., Nuclear import mechanism of neurofibromin for localization on the spindle and function in chromosome congression, *J. Neurochem.*, 2016, vol. 136, no. 1, pp. 78–91.
45. Balla, B., Árvai K., Horváth, P., Tobiás, B., Takács, I., Nagy, Z., Dank, M., Fekete, G., Kysa, J.P., and Lakatos P., Fast and robust next-generation sequencing technique using ion torrent personal genome machine for the screening of neurofibromatosis type 1 (NF1) gene, *J. Mol. Neurosci.*, 2014, vol. 53, no. 2, pp. 204–10.
46. Maruoka, R., Takenouchi, T., Torii, C., Shimizu, A., Misu, K., Higasa, K., Matsuda, F., Ota, A., Tanito, K., and Kuramochi, A., The use of next-generation sequencing in molecular diagnosis of neurofibromatosis type 1: A validation study, *Genet. Test. Mol. Biomarkers.*, 2014, vol. 18, no. 11, pp. 722–35.

Received June 22, 2018
Received September 29, 2018
Accepted May 18, 2020